



# Seagrass Sediment Sampling Protocol and Field Study: British Columbia, Washington and Oregon



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With support from the **Commission for Environmental Cooperation's** (CEC's) 2015–2016 project, *North American Blue Carbon: Next Steps in Science for Policy*.

Carbon that is stored in coastal and marine ecosystems is referred to as 'blue carbon'. Blue carbon stocks (or carbon storage) have only recently been acknowledged as globally significant (Fourqurean et al. 2012). Meta-analyses reveal that blue carbon habitats, in particular mangroves, seagrass meadows and salt marshes, play a disproportionately large role in carbon sequestration relative to their global extent, making them hot spots for carbon storage (Duarte et al. 2005; McLeod et al. 2011; Fourqurean et al. 2012). Despite their recognized importance in the global carbon budget, we have a much poorer understanding of blue carbon sinks relative to terrestrial carbon sinks (McLeod et al. 2011). This lack of understanding currently limits our ability to include blue carbon stocks in climate change strategies (Macreadie et al. 2014; Hejnowicz et al. 2015). Furthermore, the destruction and degradation of blue carbon ecosystems is concerning, as it has the potential to exacerbate the impacts of climate change, as well as limit other ecosystem services associated with these habitats (Barbier et al. 2011; Pendleton et al. 2012).



Collecting sediment cores, Pruth Bay, British Columbia.

Photo: Margot Hessing-Lewis

In order to accurately include blue carbon in global carbon budgets, it is necessary to quantify the spatial extent of blue carbon habitats as well as determine the carbon storage variability among and within these habitats. This research had two goals: to develop a method for collection and processing of sediment cores to a depth of one meter or more in eelgrass (*Zostera marina*) habitat and to provide core-based estimates of blue carbon storage and sequestration in the eelgrass beds of British Columbia, Canada and Washington and Oregon, US. Seagrass sediment blue carbon sampling was conducted at one site in British Columbia, two sites in Puget Sound, Washington, and two sites near Coos Bay, Oregon (Figure 1). Long cores (up to 100 cm long) were taken at each of these five sites. In addition, three 10 cm-long cores were collected along transects in the lower, middle and upper intertidal zones of the seagrass meadows at each site and three 10 cm-long reference cores were collected just beyond the edge of the meadows. (These smaller cores will be discussed more fully below.) Each core-sampling site is also a Seagrass-Net long-term monitoring site and all blue carbon cores were taken in conjunction with SeagrassNet monitoring.

### Coring Method Developed for British Columbia

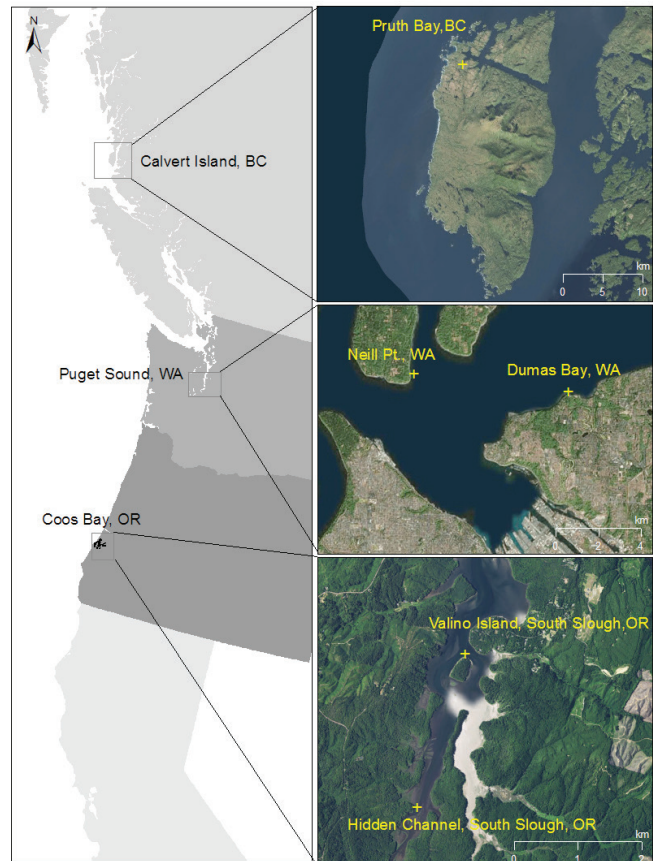
Seagrass sediment coring methods developed by the Hakai group in British Columbia were modified from TAMUCC Earth System Science Lab marsh coring methods (<http://esslab.tamucc.edu/tools-marshcoring.html>) although these were further refined for the sediment core sampling along Puget Sound, Washington, and Coos Bay, Oregon to reduce problems of sediment compaction during core insertion and to improve ease of core removal. Sediment core samplers were fabricated from schedule 40 PVC piping, with an interior diameter of 4 inches (10.16 cm). Core tubes were equipped with a steel or galvanized so-called “core catcher,” following the TAMUCC design for core tube removal.

A core catcher consists of metal “teeth” that close when the core tube is being removed from the sediments, to prevent losing sediments out the bottom of the core tube (Figure 2). A template core catcher can be used to cut new core catchers from thin metal sheets (suggested thickness is 0.010 inch, or 0.254 mm). The template can be found here: <http://esslab.tamucc.edu/resources/corecatchertemplate.pdf>.

The core catcher was inserted into the end of the core tube and secured by drilling holes through the PVC and metal and fastening with 1/8-inch rivets. Note that core catchers may only be necessary for certain sites and for longer core samples. Their usefulness in retaining sediment is site-specific, depending on sediment characteristics. The core catcher was most needed when sampling coarse sandy sediments that are common in physically dynamic environments.

Long core tubes were 150 cm in length; however, about 20 cm headspace is required at the top of the core sampler and about 10 to 15 cm of sediment is lost below the core catcher. Also, sediment may be compacted during insertion, depending on sediment type. Thus, it is important to consider sediment losses relative to the desired length of the sediment core sample when determining how long to cut the PVC sampling pipe. A rope was attached near the top of the core sampler, secured by hose clamps, to provide

Figure 1. Location of seagrass sediment core sampling sites



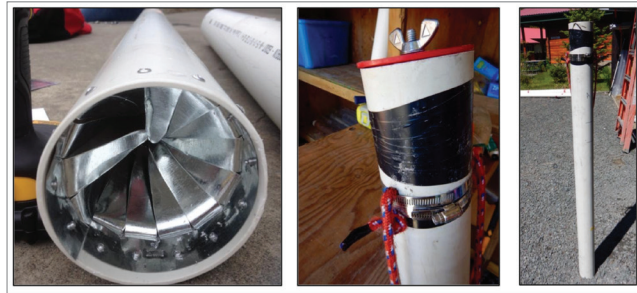
Note: Cores were obtained from one site in Pruth Bay (PB) Calvert Island, British Columbia, two sites along central Puget Sound, Washington, (WA50.1 – Dumas Bay, WA50.2 – Neill Pt.) and two sites near South Slough, Oregon (OR25.1 - Valino Island, OR HC - Hidden Creek).

a handle for removing the sediment core tube (Figure 2). Three holes were drilled approximately 2.5 cm below and around the top of the core sampler to allow water to escape if part or all of the core sampler was underwater, relieving pressure and enabling the core sampler to more easily penetrate into the sediments, ultimately decreasing sample compaction. The drilled holes were wrapped with electrical tape prior to removal from the sediment to create suction. The core tubes were pre-labeled when multiple cores were collected from the same place and time.

The samples in British Columbia were taken from May 5 to 12, 2016. Core samplers were driven into the sediments using an 8 lb. sledgehammer. A metal or wood platform was secured on top of the PVC pipe to prevent the core tube, or pipe, from cracking while being hammered and one or two people guided the core sampler vertically into the sediment. To minimize disturbance, the core sampler was pushed/pounded slowly into the sediments to the desired depth. Compaction measurements were taken during core sampler insertion to monitor how compaction rates changed with depth; compaction increases the deeper the core sampler penetrates, due to surface friction. To determine compaction, the distance was measured (cm) from the top of the core to the sediment surface on the inside of the tubing, along with the distance from the top of the core to the sediment surface on the outside of the tubing; subtracting the inside from the outside distance provided the compaction measurement. GPS location, water depth, collection time, estimated tidal height, and additional seagrass bed observations were recorded at each coring site. Note that in Oregon and Washington, in order to address compaction issues, a vibrating motor was employed to insert the core into the sediments, differing from the methodology described here.

Successful removal of the core sampler tube depended on a combination of good suction within the core, and proper activation of the core catcher. To improve suction, electrical tape was used to cover all of the vent holes at the top of the core sampler, ensuring that there was no place for air or water to escape. Then a compression cap was placed on top of the core sampler, secured by tightening a wing nut until a good seal was formed (Figure 2). A pipe or piece of wood was slipped through the rope handle and two people on either end of the wood pulled upwards to extract the core tube from its surrounding sediments. Once the suction was broken near the bottom, the core came out with minimal physical effort.

Figure 2. Details of the sediment core sampler



Note: From left to right: core catcher, rope handle for core removal, and a prepared core sampler.

Once the core sampler was successfully removed from the hole, the bottom of the tube was quickly capped to secure the sediments within. The core catcher, however, when properly installed, kept most of the sediment in place. The core sampler tubes were kept upright during transport and care was taken to minimize vibration.

The sediment cores were extruded from the sampler tubes by means of a long metal pole (>1.5 m) to which had been affixed a piston machined out of Teflon with an O-ring. A small amount of laboratory-grade vacuum grease was applied to the piston for lubrication.

The end cap and core catcher were removed from the long core tubes prior to inserting the piston into the bottom of the core sampler. The top end cap remained on to provide continued suction while removing the core catcher rivets with a drill and nail set and then securing the piston. The end cap and core catcher were taken off once the rivets were removed, and the core sampler was then swiftly and carefully lowered onto the piston, supported by two people from below and on top by a person on a ladder.

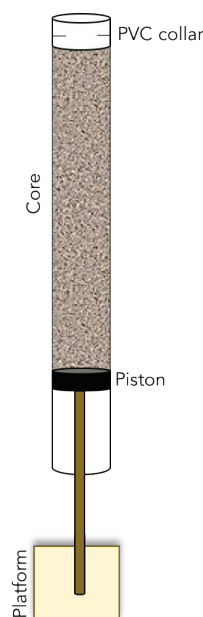
Incremental disc sections were sliced at designated intervals. Each section was measured using a collar, also called fencing, of the same diameter as the core and placed on top of the core as it was extruded from the core sampler (Figure 3). Collars of varying heights were used to obtain samples of different heights (e.g., 2 cm, 5 cm, 6 cm and 10 cm collars) at different depth intervals.<sup>1</sup>

To extrude, the core was carefully pushed down onto the piston until the sediment was even with the top of the collar. A thin piece of plastic was used to slice the section

1. Typically, the surface layer was sectioned into a disc 6 cm thick. Then, down to 20 cm of core height, the discs were of 2 cm thickness. From 20 cm to 50 cm of core height, the discs were of 5 cm thickness. From 50 cm to the bottom of the core, the discs were of 10 cm thickness.



Figure 3. Sediment core extruder setup



off of the sediment core. When the core surface was not flat at the water-sediment interface, an average height estimate was made from measurements taken around the collar.

Each depth interval sediment disc section was transferred to a pre-labeled bag and homogenized. If rocks, sticks, clamshells or other debris were found in half of one section and half in another, all the material was grouped in the section where the majority of the material was found, and a note was made of this. Additional qualitative observational notes were taken during core sectioning, including: changes in sediment composition, color, smell, grain size, as well as infauna, such as clams or worms, types of debris, or sampling obstructions.

Once each depth interval disc section was homogenized, it was analyzed for grain size, carbon content and stable isotopes, dry bulk density, and geochronology ( $^{210}\text{Pb}$  analysis). A 30 mL syringe, with the tip cut off (effectively a mini-core sampler), was used to collect 30 cc fractions of sediment for dry bulk density measurement and all chemical analyses and these fractions were each placed in a small Whirlpak bag. The remainder of the sample was kept in its original Ziploc bag for analysis of grain size and to archive extra sediments. All samples were kept on ice in a cooler during the extruding process. Sample bags were labeled with the site and location of the core, date taken, type of sample, and the section (e.g., 8–10 cm).

Wet weights (in grams or kilograms) of both bags (the 30 cc sediment fraction for chemical analyses and the larger grain-size/archived sample) for each depth interval were recorded for volumetric calculations and geochronologic sediment rate interpretations. The grain-size/archived sediment samples were stored in a refrigerator, while the 30 cc bulk density and chemical samples were kept in a freezer at  $-20^{\circ}\text{C}$ .

Additionally, at each site, six small syringe cores (10 cm in length and 30 cc in volume) were collected in conjunction with SeagrassNet monitoring. Three cores were collected along transects in the lower, middle and upper intertidal zones of the seagrass meadow, and three reference cores were collected just beyond the edge of the meadow. Syringe cores were extruded directly into labeled bags and kept in the freezer prior to processing.

### Washington and Oregon Coring Method Modifications

Sediment coring and sampling methods were improved for Washington (sampling dates: May 19 through 24, 2016) and Oregon (sampling dates: May 25 and 26, 2016), based on lessons learned during the initial sampling in British Columbia. The aim of the research was to obtain data on carbon storage for Washington and Oregon from both deep sediment cores ( $\sim 1$  m) and shallow, spatially distributed cores from seagrass beds. Seagrass blue carbon sediment sampling was conducted at two sites at Puget Sound, Washington, and two sites at Coos Bay, Oregon. Each core-sampling site is also a SeagrassNet long-term monitoring site, and blue carbon sediment cores were taken in conjunction with annual SeagrassNet monitoring. The sampling design in each meadow included both syringe cores (10 cm) along SeagrassNet transects and at reference sites (three replicate cores each) and three blue carbon sediment cores ( $\sim 1$  m) taken adjacent to the transect of the permanent SeagrassNet site.

Core samples were collected as described for British Columbia, except the core tube was inserted into the sediments using a vibrating motor (a *Milwaukee* hammer drill), which enabled the tube to be pushed into the sediment with much less compaction than occurred when pounding with a sledgehammer (see Figure 4). (Tests run in Washington during core sampler insertion on the

degree of compaction using the sledgehammer versus the vibrating motor showed typical core compaction using a sledgehammer of 18–27% and 4–10% compaction using the vibrating motor.)

Sediment core sampler tube removal was also improved over the method used in British Columbia. Before extracting the core sampler, a 5 ft. (½” diameter section of steel conduit) pipe was fitted with a loose 1” carriage bolt at the bottom of the pipe. The bolt and pipe were pushed down into the sediment along the outside of the core sampler until the top of the pipe was even with the top of the sampler. Then pulling the pipe back up a half an inch released the carriage bolt such that the pipe formed a snorkel that allowed air to break the suction at the bottom of the core. Removal of the core tube was done according to the method used in British Columbia. Likewise, core extrusion followed the same protocol. Several of the sediment cores from Washington were disrupted by rocky substrate and the presence of large shell and geoduck clams.

## Laboratory Methods and Results

The objectives of the laboratory analyses were: (1) to determine the sedimentary carbon stocks (in kg C/m<sup>2</sup>) up to 1 m below the sediment surface in seagrass and seagrass reference beds, (2) to determine the sources of sedimentary organic carbon, (3) to calculate sediment and carbon accumulation rates (g C/m<sup>2</sup>/year) for the different regions

Figure 4. Sediment coring with a hammer drill



Note: The sediment coring was greatly aided by a vibrating motor, a battery powered hammer drill, placed on a metal bar positioned on the top of the core tube. By applying pressure, the core tube was slowly and steadily inserted into the sediment with minimal compaction.

and (4) to characterize a suite of sediment characteristics that may help explain variation in organic carbon stocks.

The following methods follow the procedures outlined earlier in this report. Cores were extruded in the field and packaged into appropriately labeled bags: (a) one 30 cc sample for laboratory analyses and (b) the rest of the section in a larger bag for analyses of grain size and extra sediments. The 30 cc sediment samples were kept frozen at -20°C. Samples for analyzing grain size should be either stored in the refrigerator or freezer, but not thawed and refrozen multiple times. Samples were kept frozen during transport to the University of British Columbia (UBC) and were not removed from the freezer until preparation for the freeze-drying process. Sample processing was conducted at the University of British Columbia's Earth and Ocean Sciences, Geochemistry Lab and Forestry and Conservation Sciences, Stable Isotope Facility.

The first step in the laboratory analyses was to dry each 30 cc sample. If wet weights had not been obtained at the time the samples were collected, then they were taken prior to drying to provide information for calculations of porosity and water content. Samples were dried in an Edwards Modulyo® Freeze Dryer for approximately one week, or until fully dry. Whirlpak or Ziploc bags were opened slightly before placing them in the freeze dryer to ensure the samples dried completely. Once dry, a dry weight in micrograms was obtained for each 30 cc sample using a Mettler Toledo XP205 balance.

Once the dry weight was obtained, each sample was ground into a fine powder using a Herzog HSM 100 grinding mill. Prior to grinding, any larger particles (e.g., rocks, pieces of wood, infauna, dead plant material, shells), as well as any other general observations about the sample (e.g., very fine sediment, sandy shell-hash), were recorded. All visible living seagrass biomass (i.e., shoots, roots or rhizomes) was removed from the samples; however, we acknowledge that very small pieces of living material (e.g., small root-lets) may have been ground with the sample, particularly in the surface samples. The grinding pots were thoroughly cleaned between each sample to prevent cross-contamination. Each sample was ground for three minutes. For samples with large amounts of shell hash or coarser particles, it was necessary to split the sample up into two or three smaller samples to ensure that the sample was ground sufficiently (separate batches were re-combined and mixed

together after grinding). Fully ground samples were placed back into their respective bags.

Four different types of analyses were conducted on the ground samples. (See the table in the Appendix which lists all core samples, the 30 cc SeagrassNet and Reference samples, and the analyses performed on each.) These analyses included: (1) elemental analyses for total carbon (%C) and nitrogen (%N), (2) coulometric analyses for total inorganic carbon (%TIC), (3) stable isotope analyses for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , and (4)  $^{210}\text{Pb}$  and  $^{226}\text{Ra}$  analyses for sediment aging and subsequent determination of sediment accumulation rates.

Laboratory results showed that the percent total carbon and nitrogen was, on average, highest at the Oregon site, followed by British Columbia and then Washington. Average values  $\pm$  standard error, combining all long and syringe cores in each region, were  $1.31 \pm 0.053$ ,  $0.67 \pm 0.060$  and  $0.32 \pm 0.025$  percent carbon and  $0.08 \pm 0.004$ ,  $0.06 \pm 0.005$  and  $0.02 \pm 0.001$  percent nitrogen for Oregon, British Columbia and Washington, respectively.

The sediment carbonate content and percent inorganic carbon was overall quite low (<1% in almost all cases), but for some samples, specifically at the Washington site, total carbon was also quite low and thus inorganic carbon accounted for 20 to 60% of the total carbon. Typically, large total inorganic carbon (TIC) values were seen where bivalve shells were observed within the sections. Average %TIC values  $\pm$  standard error, combining all long and syringe cores in each region, were  $0.017 \pm 0.003$ ,  $0.099 \pm 0.024$  and  $0.068 \pm 0.015$  for Oregon, British Columbia and Washington, respectively. This made up, on average, 1.3, 14.7 and 21% of the total carbon for Oregon, British Columbia and Washington, respectively.

For every subsection of each core, the percent organic carbon was calculated by subtracting the percent inorganic carbon from the percent total carbon. Average percent organic carbon values  $\pm$  standard error, combining all long and syringe cores in each region, were  $1.285 \pm 0.053$ ,  $0.569 \pm 0.051$  and  $0.248 \pm 0.053$  for Oregon, British Columbia and Washington, respectively.

All sediments measured were predominantly fine sand, with the Oregon Hidden Creek site (OR HC) sediment surface having the most mud (25–46%) and the least coarse sand (26%). The greatest percent sand was at the WA50.2, BC and OR 25.1 site and the sand fraction was

the same. WA50.1 had the lowest mud content (2%) and the greatest amount of coarse sand (35%).

The average bulk density of the eelgrass sediments for the five sites ranged from  $\sim 1.0$  to  $1.6 \text{ g/m}^3$  and showed no consistent pattern with depth in the core. Wet and dry weights for the 30 cc samples were used to calculate sediment dry bulk density and percent moisture. Site OR HC in Hidden Creek had lower bulk density than all the other sites. The sediment carbon density ( $\text{mg C m}^{-3}$ ) was calculated from bulk density and sediment carbon content (Figure 5).

For  $\delta^{13}\text{C}$  the OR values were on average most negative, followed by WA and BC, which were slightly less negative (Figure 6); the average  $\pm$  standard error values were  $-23.19 \pm 0.275$ ,  $-19.48 \pm 0.863$  and  $-16.23 \pm 0.635$  for OR, WA and BC, respectively. The range across all samples and sites was from  $-11.94$  to  $-24.26\text{‰}$   $\delta^{13}\text{C}$ . For the  $\delta^{15}\text{N}$  values, the BC values were the most positive, followed by Oregon and then Washington; the average  $\pm$  standard error values were  $7.18 \pm 0.201$ ,  $6.94 \pm 0.125$  and  $6.80 \pm 0$  for BC, OR and WA, respectively. Average values were similar across samples and sites, with a total range of 6.3 to 8.2‰  $\delta^{15}\text{N}$ .

For the three locations,  $^{13}\text{C}$  and  $^{15}\text{N}$  isotopes in eelgrass sediments clearly show distinct sources of carbon, while only BC and WA show differences in nitrogen sources. Seagrass  $^{13}\text{C}$  values for WA and BC are comparable to those of other eelgrass sediments (Rohr et al. 2016). The  $^{13}\text{C}$  value for the OR site is significantly lower than those for WA and BC (Figure 6).

Using the sediment mass accumulation rate derived from the  $^{210}\text{Pb}$  results, with the organic carbon fraction, we determined the carbon accumulation rates at different depths for five long cores. (See  $^{210}\text{Pb}$  column of the table in the Appendix.) The accumulation of sediments at the British Columbia and the two Oregon sites as measured by  $^{210}\text{Pb}$  dating were different between sites and appeared related to the extent of eelgrass at various times at the site. The British Columbia site showed a low to steady sediment accumulation in what is now a moderate cover eelgrass bed, but there was rapid accumulation from the 1950s through the 1990s, followed by an interval of very high accumulation around 2004 followed by a decline through 2016. At the site in Hidden Creek, OR (OR HC), there was moderate sediment accumulation where sparse

Figure 5. Carbon density in sediment cores of the BC, WA, and OR sites

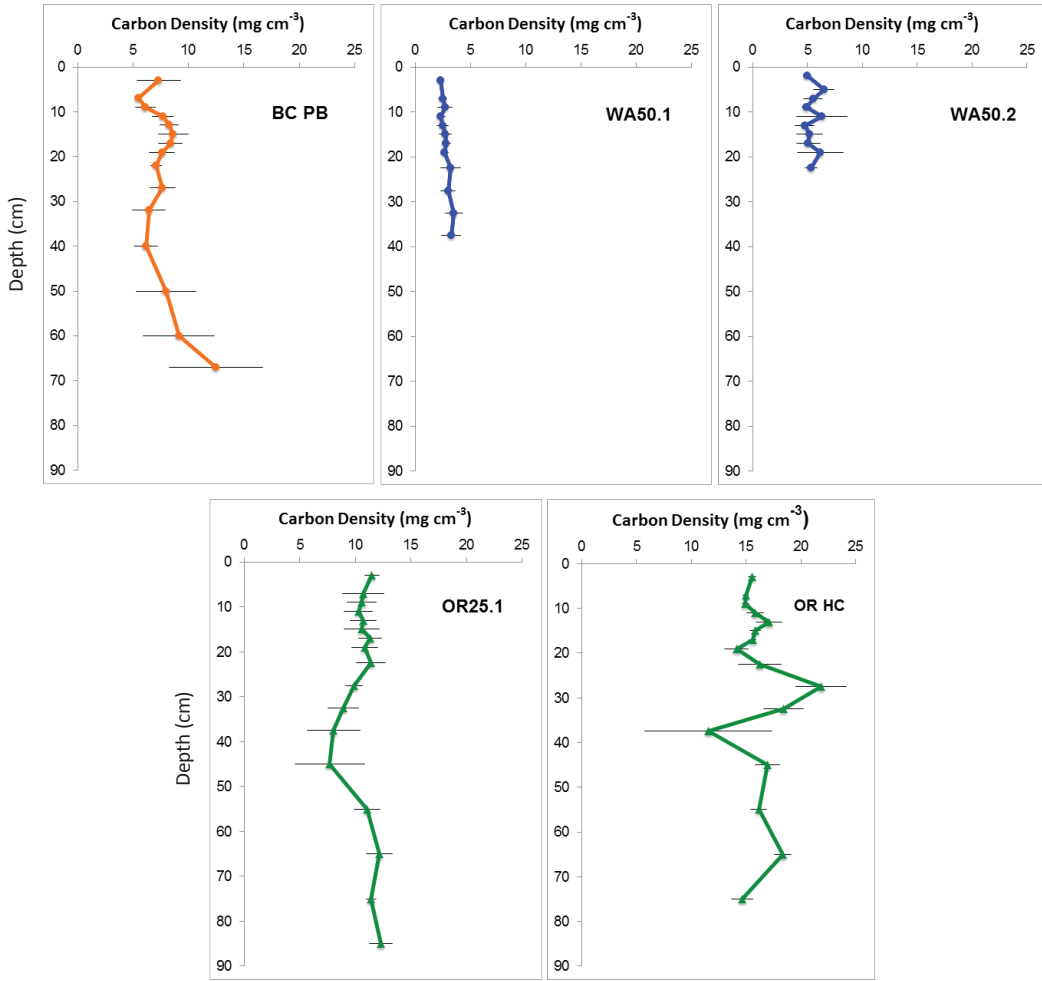
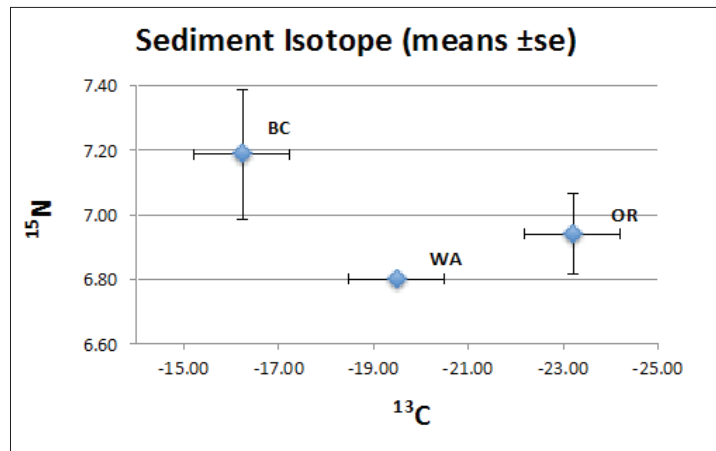


Figure 6. Carbon (<sup>13</sup>C) and nitrogen (<sup>15</sup>N) isotope data for the eelgrass sediments at BC, WA, and OR sites





eelgrass occurred in 2016. At the South Slough Seagrass-Net site, OR 25.1, there is high sediment accumulation in a dense eelgrass bed; here, sediment has accumulated at an increasing rate since the early 1900s.

Carbon accumulation followed the overall rate of sediment accumulation. Comparing carbon storage in the upper 18 cm versus the upper 10 cm of all cores, the same carbon accumulation pattern was seen, with the 2 Washington

cores having the least carbon storage and Hidden Creek in Oregon (2 cores) having the greatest amount (Table 1). Between the OR and BC sites, the rate of accumulation of carbon was lowest at the British Columbia site (1 core) and greatest at OR 25.1. It was impossible to get dating information or carbon accumulation rates from the cores at the Washington sites because  $^{210}\text{Pb}$  was at background levels and no signal could be detected.

**Table 1. Eelgrass percent cover, carbon stocks, and carbon accumulation rates for the BC and two Oregon sites, based on long cores at each site ( $\text{t C km}^{-2} \text{y}^{-1} = \text{g C m}^{-2} \text{y}^{-1}$ )**

Site	Eelgrass Cover %	Carbon Stock (top 18 cm) $\text{kg C m}^{-2}$	Carbon Stock (top 10 cm) $\text{kg C m}^{-2}$	Carbon Accumulation Rate (top 10 cm) $\text{g C m}^{-2} \text{y}^{-1}$
BC PB	65	1.37	0.62	7.8
WA 50.1	34	0.42	0.22	-
WA 50.2	10	0.71	0.27	-
OR 25.1	70	2.35	1.32	32.4
OR HC	25	2.81	1.56	26.4



Eelgrass meadow, Pruth Bay, British Columbia.



## Conclusions

Using the protocol for collection of deep sediment cores in eelgrass (*Zostera marina*) sediments described above, the sequestration and storage of carbon in eelgrass sediment was assessed in North America's Pacific Northwest by collecting deep sediment cores from British Columbia, Washington and Oregon eelgrass beds. The carbon content of the eelgrass sediments was fairly consistent between replicate cores, but differed between the states and province as well as between individual sites. In Oregon, the Hidden Creek site, OR HC, stands out as having substantially more carbon in the sediment throughout its depth profiles than any of the other sites studied. The carbon content of surface layers observed in the five cores in our study ranges between 0.16 and 1.92%. The carbon content of the eelgrass surface sediments is in the same range as those found in eelgrass meadows of Finland and Denmark (Röhr et al. 2016).

Bulk density is a measure of the weight of the non-water portion of the sediments per unit volume. Low bulk density is an indicator of higher water content. When the bulk density is known, the portion of carbon per volume of sediments can be calculated. Sediments that are higher in carbon hold more water and thus have lower bulk density. The sediment dry bulk densities measured in British Columbia, Washington and Oregon are typical of other eelgrass areas (Rohr et al. 2016), although that of the Oregon OR HC site is lower than the other sites in our study. The depth profiles of bulk density demonstrate a different sedimentary environment over the last century at the OR HC site than our other sites. The sediment grain size data for the different sites clearly show the muddier sediments of the OR HC site, located in a remote channel (Figure 1), within eelgrass as well as in non-vegetated reference areas.

The carbon density, calculated from the bulk density and its carbon fraction, presents a useful measure of the carbon distribution through the sediment profile (Figure 5). These profiles show clearly the average amount of carbon at the different coring sites as well as in the sediment layers at each site. The Hidden Creek, Oregon site had the highest carbon density over the length of the core, averaging  $16 \text{ mg C m}^{-3}$ . In contrast, the lowest values were at the WA50.1 site, which had carbon density values averaging  $2.8 \text{ mg C m}^{-3}$  throughout the depth of the core.

$^{210}\text{Pb}$  dating of a sediment core from each site allowed the assessment of sediment accumulation rates. The age of the core sections was determined by measuring the amount of excess  $^{210}\text{Pb}$  over the background Pb isotopes. Analysis of sediment at the Oregon and British Columbia sites shows that the rate of accumulation is higher at present than in the recent past. At these locations, the maximum core depth extended to the 1950s for BC, 1930s for OR HC, and to 1910 for OR25. For Puget Sound, Washington, the excess  $^{210}\text{Pb}$  content of the sediment was too low to obtain dates from the sediment cores, likely due to the high sand content of the sediments (>95%) and low organic carbon content (0.02%). We calculated the rate of carbon accumulation and found the greatest C accumulation rate at OR25.1 of  $32.4 \text{ g C m}^{-2} \text{ y}^{-1}$ , and the lowest carbon accumulation rate of  $7.8 \text{ g C m}^{-2} \text{ y}^{-1}$  for the BC site (Table 1). The associated carbon storage in the upper 18 cm of sediments for these three sites is 1.4, 2.4 and  $2.8 \text{ kg C m}^{-2}$  for BC, OR HC and OR25.1, respectively. The carbon storage for the comparable sediment depths for Washington was considerably lower, with 0.43 and  $0.71 \text{ kg C m}^{-2}$  for WA50.1 and WA50.2, respectively.

The carbon sequestration rates (accumulation rates) and carbon storage values provide some actual measures of blue carbon capacity for the three locations, but are not adequate to be representative of entire regions. More research is needed in different eelgrass habitat types and across these locations to provide a representative range of values. A number of concerns need to be considered in future research to improve sediment carbon measurements: the compaction of cores from vigorous insertion of the tube into the sediments can be partly alleviated by using the vibrating hammer rather the sledge hammer, and partly by not forcing the core to penetrate the under-burden below the eelgrass sediments; the loss of pore water draining out of the core after collection which affects moisture content measures and bulk density measures; shells, rocks, and clams in the core.

Stable isotope analyses of eelgrass sediments were conducted to get an idea about where the sediment carbon originated. Comparison of nitrogen (N) isotopes to C allows examination of source materials and shows distinct  $^{13}\text{C}$  sources occurring in the different locations. The separation between  $^{15}\text{N}$  values is much less. To understand what possible sources may be involved in the different locations, the isotope data from our analysis was overlaid on a source domain plot derived from Cloern et al. (2002).

Oregon sediment organic matter is mostly eelgrass origin with likely augmentation by salt marsh C4 plants; the Washington site sediments on the exposed shore of Puget Sound are likely a mix of eelgrass, phytoplankton, and algal material; and the British Columbia site is predominated by eelgrass, C4 marsh plants and phytoplankton. The  $^{15}\text{N}$  isotope distribution from the three sites indicates all are at the lower range of eelgrass values, suggesting that these eelgrass beds are not substantially impacted by anthropogenic N sources. The  $^{13}\text{C}$  excess values for British Columbia and Washington were in the range of sites sampled in the Baltic (Röhr et al. 2016), but the Oregon sites, in a slough surrounded by salt marsh, were slightly higher and had higher  $^{13}\text{C}$  values.

Results show that eelgrass habitat sequesters and stores carbon not only from the eelgrass itself but also from adjacent surrounding habitat. Oregon had the greatest C storage per square meter and the greatest rates of sequestration of the three locations, with British Columbia being half of that and Washington half again. However, given the different character of these sites, with very different sediment grain sizes, organic carbon sources, and apparent wave exposure conditions, it is likely the differences seen between BC, WA and OR are not a result of geographic separation. Thus, our findings suggest that this geographic region has a minimum storage of 0.4 to 2.8 kg C m<sup>-2</sup> and a minimum sequestration rate of 7.8 to 32.4 g C m<sup>-2</sup> y<sup>-1</sup>. Our sequestration findings are comparable to the K'ómoks Estuary, BC, with 22.8 g C m<sup>-2</sup> y<sup>-1</sup> (Hodgson and Spooner 2016) and those from Finland, 5.2 g C m<sup>-2</sup> y<sup>-1</sup>, and Norway, 35.2 g C m<sup>-2</sup> y<sup>-1</sup> (Röhr et al. 2016).

The sampling methods described here using inexpensive coring tubes, fabricated extrusion devices, a vibrating motor for non-compacting core penetration, and a snorkel-like tube for extraction, produced results that captured carbon conditions in a range of Pacific Northwest eelgrass environments. Using them, we provided some preliminary measures of blue carbon data for this region and calculated blue carbon storage and carbon sequestration.

## Acknowledgments

Thanks to the many scientists and volunteers who contributed to this project: Dante Torio (University of New Hampshire, USA), scientists, staff and volunteers from the Hakai Institute (West Coast, BC), University of British Columbia, Washington State Department of Natural Resources, Western Washington University, and the South Slough National Estuarine Research Reserve (OR). We especially thank Cathy Short for writing and editing.

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## Appendix: Cores Collected and Analyzed

Region	Cores	Total Length of Core (cm)	Number of Subsections	Total Carbon	Total Inorganic Carbon	<sup>210</sup> Pb Dating	Stable Isotopes
British Columbia	Pruth Bay A	80	16	✓	✓	✓	✓
	Pruth Bay B	74	16	✓	✓		✓
	Pruth Bay C	70	15	✓	✓		✓
	SeagrassNet	10	0 (3 reps)	✓	✓		✓
	Reference	10	0 (3 reps)	✓	✓		✓
Washington	50.1-1	57	16	✓	✓	✓	✓
	50.1-2	57	16	✓	✓		✓
	50.1-3	40	12	✓	✓		✓
	50.2-1	10	4	✓	✓		✓
	50.2-2	25	10	✓	✓		✓
	50.2-3	25	10	✓	✓	✓	✓
	SeagrassNet	10	0 (3 reps)				
	Reference	10	0 (3 reps)	✓	✓		✓
Oregon	HC-1	100	18	✓	✓	✓	✓
	HC-2	78	16	✓	✓		✓
	HC-3	100	18	✓	✓		✓
	SeagrassNet	10	0 (2 reps)	✓	✓		✓
	Reference	10	0 (3 reps)	✓	✓		✓
	25.1-1	90	17	✓	✓	✓	✓
	25.1-2	105	19	✓	✓		✓
	25.1-3	100	18	✓	✓		✓
	SeagrassNet	10	0 (3 reps)	✓	✓		✓
	Reference	10	0 (3 reps)	✓	✓		✓